

We Claim:

- ✓
1. A method for reverse transcribing an RNA, that comprises:
- (a) providing a reverse transcription reaction mixture comprising said RNA, a primer, a divalent cation, and a mutant thermoactive DNA polymerase, wherein said mutant DNA polymerase is characterized in that
- 5 i) in its native form said DNA polymerase comprises an amino acid sequence that is SEQ ID NO: 1;
- ii) the amino acid at position 4 of said amino acid sequence is mutated in comparison to said native sequence to an amino acid other than E, A, G, or P; and
- 10 (b) treating said reaction mixture at a temperature sufficient for said mutant DNA polymerase to initiate synthesis of an extension product of said primer to provide a cDNA molecule complementary to said RNA.
- 15 2. The method of Claim 1, wherein said amino acid sequence is SEQ ID NO: 2.
3. The method of Claim 1, wherein said amino acid sequence is SEQ ID NO: 3.
4. The method of Claim 1, wherein said amino acid sequence is SEQ ID NO: 4.
- 20 5. The method of Claim 1, wherein said amino acid sequence is SEQ ID NO: 5.
6. The method of Claim 1, wherein said amino acid sequence is SEQ ID NO: 6.
- 25 7. The method of Claim 1, wherein said amino acid sequence is SEQ ID NO: 7.
8. A method of Claim 1, wherein said mutant DNA polymerase is thermostable.
9. The method of claim 1, wherein said DNA polymerase is a mutant form of a
- 30 *Thermus* species DNA polymerase.
10. The method of claim 1, wherein said DNA polymerase is a mutant form of *Thermus thermophilus* DNA polymerase or *Thermus aquaticus* DNA polymerase.

11. The method of Claim 1, wherein said temperature of said reaction mixture in step (b) is between 40°C and 80°C.

12. The method of Claim 1, wherein said amino acid at position 4 of said amino acid sequence is mutated in comparison to said native sequence to an amino acid other than E, A, G, P, Q, or D.

✓ 13. A method for reverse transcribing an RNA, that comprises:

(a) providing a reverse transcription reaction mixture comprising said RNA, a primer, Mg^{+2} , and a mutant thermoactive DNA polymerase, wherein said mutant DNA polymerase is characterized in that

i) in its native form said DNA polymerase comprises an amino acid sequence that is SEQ ID NO: 1;

ii) the amino acid at position 4 of said amino acid sequence is mutated in comparison to said native sequence to an amino acid other than E, A, G, or P; and

(b) treating said reaction mixture at a temperature sufficient for said mutant DNA polymerase to initiate synthesis of an extension product of said primer to provide a cDNA molecule complementary to said RNA.

20 14. The method of Claim 13, wherein said amino acid sequence is SEQ ID NO: 2.

15. The method of Claim 13, wherein said amino acid sequence is SEQ ID NO: 3.

25 16. The method of Claim 13, wherein said amino acid sequence is SEQ ID NO: 4.

17. The method of Claim 13, wherein said amino acid sequence is SEQ ID NO: 5.

18. The method of Claim 13, wherein said amino acid sequence is SEQ ID NO: 6.

30 19. The method of Claim 13, wherein said amino acid sequence is SEQ ID NO: 7.

20. The method of Claim 13, wherein said mutant DNA polymerase is thermostable.

21. The method of Claim 13, wherein said DNA polymerase is a mutant form of a *Thermus* species DNA polymerase.

22. The method of Claim 13, wherein said DNA polymerase is a mutant form of
5 *Thermus thermophilus* DNA polymerase or *Thermus aquaticus* DNA polymerase.

23. The method of Claim 13, wherein said temperature of said reaction mixture in step (b) is between 40°C and 80°C.

10 24. The method of Claim 13, wherein said amino acid at position 4 of said amino acid sequence is mutated in comparison to said native sequence to an amino acid other than E, A, G, P, Q, or D.

25. A method for amplifying an RNA, that comprise:
15 (a) reverse transcribing said RNA according to a method of Claim 1 to provide a cDNA;
(b) amplifying said cDNA.

26. A method of Claim 25, wherein in step (b) said amplifying is carried out using a
20 polymerase chain reaction.

27. A method for amplifying an RNA, that comprise:
(a) reverse transcribing said RNA according to a method of Claim 13 to provide a cDNA;
25 (b) amplifying said cDNA.

28. A method of Claim 27, wherein in step (b) said amplifying is carried out using a polymerase chain reaction.

✓ 29. A method for amplifying an RNA using a single-enzyme reverse transcription/amplification reaction, that comprises:

(a) providing an amplification reaction mixture comprising said RNA, a pair of primers, a divalent cation, and a mutant thermostable DNA polymerase, wherein said mutant DNA polymerase is characterized in that

i) in its native form said DNA polymerase comprises an amino acid sequence that is SEQ ID NO: 1;

ii) the amino acid at position 4 of said amino acid sequence is mutated in comparison to said native sequence to an amino acid other than E, A, G, or P; and

(b) treating said reaction mixture at a temperature sufficient for said mutant DNA polymerase to initiate synthesis of an extension product of said primer to provide a cDNA molecule complementary to said RNA;

(c) treating said reaction mixture at an appropriate temperature for said mutant DNA polymerase to initiate synthesis of an extension product of said second primer to provide a double-stranded cDNA molecule; and

(d) amplifying said double-stranded cDNA molecule of step (c) by a polymerase chain reaction.

30. The method of Claim 29, wherein said amino acid sequence is SEQ ID NO: 2.

31. The method of Claim 29, wherein said amino acid sequence is SEQ ID NO: 3.

32. The method of Claim 29, wherein said amino acid sequence is SEQ ID NO: 4.

33. The method of Claim 29, wherein said amino acid sequence is SEQ ID NO: 5.

34. The method of Claim 29, wherein said amino acid sequence is SEQ ID NO: 6.

35. The method of Claim 29, wherein said amino acid sequence is SEQ ID NO: 7.

36. The method of Claim 29, wherein said mutant DNA polymerase is thermostable.

37. The method of Claim 29, wherein said DNA polymerase is a mutant form of a *Thermus* species DNA polymerase.

38. The method of Claim 29, wherein said DNA polymerase is a mutant form of *Thermus thermophilus* DNA polymerase or *Thermus aquaticus* DNA polymerase.

5 39. The method of Claim 29, wherein said temperature of said reaction mixture in step (b) is between 40°C and 80°C.

40. The method of Claim 29, wherein said amino acid at position 4 of said amino acid sequence is mutated in comparison to said native sequence to an amino acid other than E, A, G, P, Q, or D.
10

✓ 41. A method for amplifying an RNA using a single-enzyme reverse transcription/amplification reaction, that comprises:

(a) providing an amplification reaction mixture comprising said RNA, a pair of
15 primers, Mg^{+2} , and a mutant thermostable DNA polymerase, wherein said mutant DNA polymerase is characterized in that

i) in its native form said DNA polymerase comprises an amino acid sequence that is SEQ ID NO: 1;

ii) the amino acid at position 4 of said amino acid sequence is mutated in
20 comparison to said native sequence to an amino acid other than E, A, G, or P; and

(b) treating said reaction mixture at a temperature sufficient for said mutant DNA polymerase to initiate synthesis of an extension product of said primer to provide a cDNA molecule complementary to said RNA;

(c) treating said reaction mixture at an appropriate temperature for said mutant
25 DNA polymerase to initiate synthesis of an extension product of said second primer to provide a double-stranded cDNA molecule; and

(d) amplifying said double-stranded cDNA molecule of step (c) by a polymerase chain reaction.

30 42. The method of Claim 41, wherein said amino acid sequence is SEQ ID NO: 2.

43. The method of Claim 41, wherein said amino acid sequence is SEQ ID NO: 3.

44. The method of Claim 41, wherein said amino acid sequence is SEQ ID NO: 4.
- 5 45. The method of Claim 41, wherein said amino acid sequence is SEQ ID NO: 5.
46. The method of Claim 41, wherein said amino acid sequence is SEQ ID NO: 6.
47. The method of Claim 41, wherein said amino acid sequence is SEQ ID NO: 7.
- 10 48. The method of Claim 41, wherein said mutant DNA polymerase is thermostable.
49. The method of Claim 41, wherein said DNA polymerase is a mutant form of a *Thermus* species DNA polymerase.
- 15 50. The method of Claim 41, wherein said DNA polymerase is a mutant form of *Thermus thermophilus* DNA polymerase or *Thermus aquaticus* DNA polymerase.
51. The method of Claim 41, wherein said temperature of said reaction mixture in step (b) is between 40°C and 80°C.
- 20 52. The method of Claim 41, wherein said amino acid at position 4 of said amino acid sequence is mutated in comparison to said native sequence to an amino acid other than E, A, G, P, Q, or D.